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Period2 gene deletion abolishes αMelanocyte stimulating hormone response to ethanol

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PERIOD2 GENE DELETION ABOLISHES αMELANOCYTE STIMULATING HORMONE RESPONSE TO ETHANOL

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INTRODUCTION

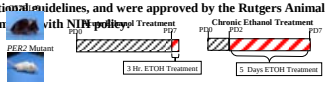
Proopiomelanocortin (POMC)-producing neurons in the arcuate nucleus of the hypothalamus secrete αMSH (1). Studies have showed that αMSH plays an important role in the regulation of several biological functions including a role in physiological responses to drug of abuse (2). There is accumulating evidence that the neuropeptide αMSH modulates neurobiologic responses to ethanol. First, αMSH is expressed in brain regions implicated in ethanol's effects (3, 4). Second, chronic exposure to ethanol significantly reduced, while abstinence following chronic ethanol exposure increased, endogenous αMSH immunoreactivity in specific brain regions of Sprague-Dawley rats (4, 5). Our lab has showed that in adults and fetal rats, alcohol exposure alters POMC gene expression and the β-endorphin peptide release from the hypothalamus (6,7). Since, both β-endorphin and αMSH peptides are produced from POMC precursor gene, the possibility arose that the level of αMSH in the hypothalamus is similarly altered by alcohol exposure. Thus, in this study we used the neonatal mice model to determine whether acute or chronic alcohol exposures alter levels of αMSH and POMC mRNA in the hypothalamus.

Recently, an interaction between αMSH and a clock gene *Period* 2 *(Per2)* has been demonstrated in the hypothalamus. Yang *et al.* (2009) demonstrated that mPer2 suppresses feeding during the inactive period by regulating the circadian rhythm of αMSH in the hypothalamus (8). In addition, our lab provided evidence that alcohol feeding in fetuses and adults alters circadian rhythms of *Period* genes (*Per* 1, 2, 3) in the hypothalamus (6,7). Clinical studies revealed that alcoholics with a specific set of polymorphisms in the *Per*2 gene consume less alcohol than alcoholics without the polymorphisms (9). Hence, *Per*2 appears to be a targeted gene where alcohol may act on to alter circadian functions. In order to elucidate the role of *Per*2 gene in modulation of neurobiological responses to ethanol, we examined the effect of *Per*2 mutation on hypothalamic αMSH neuronal responses to acute and chronic ethanol.

MATERIALS AND METHODS

Animals Methods

To study the effect of both acute and chronic ethanol administration on αMSH level of hypothalamic neurons of control and Per2 knockout mice. The Pregnant C57BL/6 and Per 2 mutant mice were individually housed in 12 hour light/12 hour dark cycles. The newborn mice (C57BL/6 and Per 2 mutant mice) were treated with ethanol in the following way: the acute ethanol exposure animals were only given treatment on day PD7, whereas the chronic ethanol exposure animals received treatment from PD2-PD7. At day of treatment two pups from each litter were fed by intubation with milk formula containing 11.34% (vol/vol) alcohol (alcohol fed - AF), a solution (0.1-.2 ml/animal; during a period of 1 minute) containing ethanol and milk formula, yielding a total daily ethanol dose of 2.5g/kg; or an isocaloric volume of maltose dextrin (pair fed - PF) as in Goodlet et al., (1998); or leave alone (ad libitum - AD). The feeding was conducted at 1000 and 1200 h daily. After feeding, the pups were immediately returned to the litter. One hour after the last feeding, brains were collected and the medial basal hypothalamic tissue was divided into halves. One half was used for gene expression analyses and the other was used to measure αMSH levels in the hypothalamus. Six animals per treatment were used (a total of 18 animals were used per experiment). The samples were stored accordingly. Animal care and treatment were performed in accordance with institutional guidelines, and were approved by the Rutgers Animal Care and facilities Committee and con[illegible] with NIH policy.



αMSH immune assay

The α melanocyte stimulating hormone (αMSH) assay (EK-043-01) was purchased from Phoenix Pharmaceuticals, Inc. (Burlingame, CA). The assay was prepared according to the manufacture's protocol. The calculation was based according to the manufacture's protocol. The samples were normalized to total μg/ml protein.

Real time Reverse transcriptase-polymerase chain reaction

The total RNA was isolated from the hypothalamic tissue of each treatment group (Ad libitum, pair-fed, and alcohol-fed) by using the trizol plus RNA purification system (Invitrogen, CA). Then the high-capacity cDNA reverse transcription kit from applied Biosystems (Foster City, CA) was used for the RT reaction. The cDNA was subjected to real-time PCR on an ABI Prism 7500 sequence detector (Applied Biosystems, Foster City, CA). The POMC primer were acquired from Applied Biosystems (Foster City, CA).

Statistics

Quantitative results are indicated as mean ± SEM. Data obtained in the studies dealing with ethanol effects on α MSH level of each strain were compared using one-way ANOVA followed by Newman Keuls post-hoc test. The ethanol effects between Per2 mutant and wildtype mice were assessed with two-way ANOVA with post hoc analysis using the Bonferroni post-test. A value of $p < 0.05$ was considered significant.

Comparing the response of hypothalamic α melanocyte stimulating hormone (α-MSH) levels in C57BL/6 and *Per*2 mutant mice upon acute and chronic ethanol (ETOH) exposures

Figure A

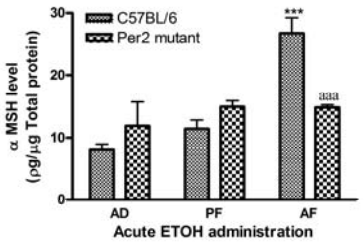


Figure B

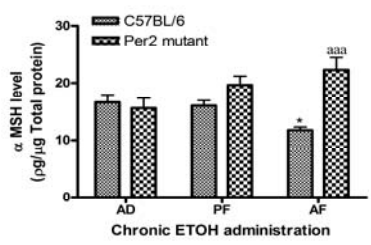


Figure 1. Effects of ethanol administration on α MSH levels in mediobasal hypothalami of C57BL/6 and Per2 mutant mice. A. Postnatal mice were fed milk formula containing ethanol (AF) or no ethanol (PF) or left in the litter (AD) for 3h. Data are ± SEM of six independent observations. ***P < 0.001, significantly different from controls of the same strain. [aaa] P < 0.001, significantly different between two strains at the same dose. B. Postnatal mice were fed milk formula containing ethanol (AF) or no ethanol (PF) or left in the litter (AD) for 5 days. Data are ± SEM of six independent observations. *P < 0.05, significantly different from controls of the same strain. [aaa] P < 0.001, significantly different between two strains at the same dose.

Comparing the response of proopiomelanocortin (POMC) mRNA levels in C57BL/6 and *Per*2 mutant mice upon acute and chronic ethanol (ETOH) exposures

Figure A

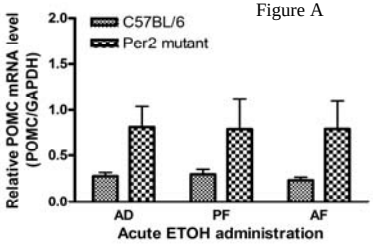


Figure B

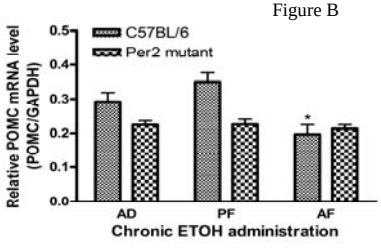


Figure 2. Effects of ethanol administration on POMC gene expression in mediobasal hypothalami of C57BL/6 and Per2 mutant mice. A. Postnatal mice were fed milk formula containing ethanol (AF) or no ethanol (PF) or left in the litter (AD) for 3 h. Data are ± SEM of six independent observations. B. Postnatal mice were fed milk formula containing ethanol (AF) or no ethanol (PF) or left in the litter (AD) for 5 days. Data are ± SEM of six independent observations. *P < 0.05, significantly different from controls of the same strain.

RESULTS

1. In this study we showed that C57BL/6 mice acutely exposed to ethanol exhibited an increase in αMSH levels in the hypothalamus, however no response to ethanol was seen in Per2 mutant mice.
2. Chronic exposure to ethanol reduced αMSH levels in hypothalami of C57BL/6 mice but not in Per2 mutant mice .
3. The POMC gene expression in both C57BL/6 and Per2 mutant mice were not altered upon acute ethanol treatment.
4. Under chronic ethanol exposure, C57BL/6 mice showed a significant reduction in POMC expression whereas Per2 mice did not show any response to ethanol.

CONCLUSION

In light of the above observations, the data support the involvement of Per2 gene in mediating the POMC neurons response to ethanol.

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